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Glycerogalactolipids from the fruit of *Lycium barbarum*

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ABSTRACT

Four glycerogalactolipids (**1–4**), together with 11 other previously known homologues were isolated from the fruit of *Lycium barbarum*. Their structures were elucidated by chemical analyses including regio-selective enzymatic, alkaline and acidic hydrolyses and spectroscopic methods involving GCMS, HRESIMS and 1D and 2D NMR, respectively.

1. Introduction

Lycium barbarum L. (Solanaceae) is distributed from the south-east of Europe to China (Mabberley, 2000). It is a well known traditional Chinese medicine, recorded as Gouqizi in the Chinese pharmacopoeia (The Pharmacopoeia Commission, 2005). Goji berries have a long history of use for the treatment of eye problems, skin rashes, psoriasis, allergies, insomnia, chronic liver disease, diabetes, tuberculosis, and kidney disorders. There are many ways that people consume this fruit for example; eating raw, drinking juice and/or smoothies, mixed with tea, and added to trail mix, cereals, muffins, energy bars or soups. The pharmacological activities associated with *L. barbarum* include hypoglycemic, immunomodulation, anti-hypertension, lipotropic, protecting hepatic function, anti-aging, anti-fatigue, antioxidant and so on (Yu et al., 2006). In spite of a number of phytochemical and bioactivity related reports on saccharides of *L. barbarum* fruit (Yoshiko et al., 2004; Lin et al., 2008), its non-polar constituents have been rarely explored. A comprehensive phytochemical investigation of the non-polar constituents of Goji berries was carried out as part of our program to identify chemical and/or biomarkers of the dietary supplements. In this report, the isolation and characterization of four new and 11 known glycerogalactolipids are described. Their structures were determined by chemical methods including regio-selective enzymatic, alkaline and acidic hydrolyses and spectroscopic analyses involving GCMS, HRESIMS and 1D and 2D NMR.

2. Results and discussion

The sugar constituents of the concentrated methanolic extract of Goji berries were removed by precipitation with acetonitrile. Fifteen glycerogalactolipids were separated from the acetonitrile soluble part by repeated column chromatography over normal and reversed-phase (RP-C_{18}) silica gel. Compound **1** was obtained as a colorless gum. The absorption bands observed at 3380 and 1737 cm^{-1} in its IR spectrum indicated the presence of hydroxyl and ester functions. The quasimolecular ion in the negative HRESIMS of **1** at *m/z* 1209.7792 $[M + Cl]^-$ corresponded to the molecular formula of $C_{67}H_{114}O_{16}$. The ^{13}C NMR spectrum showed five well differentiated groups of resonances between δ_C 10–40 (alkyl chain), δ_C 60–75 (13 methine and methylene carbons of saccharides and glycerol), δ_C 101.2 and 105.7 (two anomeric carbons), δ_C 125–135 (12 olefinic carbons), and about δ_C 174 (three acid carbonyl carbons). The oxygenated carbon resonances at δ_C 63.7 (CH_2), 71.2 (CH) and 68.3 (CH_2) suggested a glycerol moiety. The above spectroscopic data indicated that **1** was a glyceroglycolipid containing a disaccharide moiety and three fatty acid units. The olefinic resonances centered at δ_H 5.49 in the ^1H NMR spectrum can be attributed to the isolated double bonds in the fatty acid residues. An intense multiplet at about δ_H 1.25 corresponded to the aliphatic methylene protons, whereas the resonances at δ_H 4.0–6.0 accounted for disaccharide and glycerol moieties. The ^1H and ^{13}C NMR spectroscopic data assignment of **1** was facilitated by comparison with those of identical published compounds (Reshef

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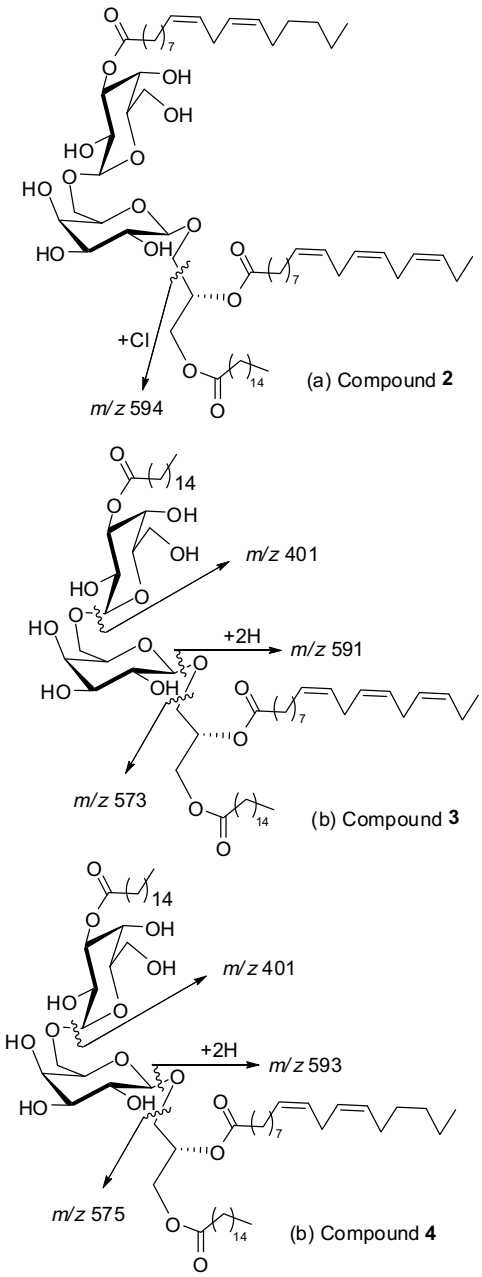
et al., 1997; Jung et al., 1996; Murakami et al., 1991) and confirmed by ^1H–^1H COSY, HMQC and HMBC spectra. The down-field shifted C-6′ methylene (δ_C 68.3) indicated the C-1″-C-6′ linkage of the sugar units (Murakami et al., 1991). The down-field shift of H-3″ (δ_H 5.83) and C-3″ (δ_C 75.3) and the up-field shift of C-2″ (δ_C 68.7) and C-4" (δ_C 67.8), when compared with those of the glycerodigalactolipids with no acyl unit at galactose (Murakami et al., 1991; Reshef et al., 1997; Jung et al., 1996), suggested the third fatty acid unit at C-3″. The sugar units were identified as D-galactose by GC analysis of their acetylated thiazolidine derivatives (Hara et al., 1987; Ali and Khan, 2008). The β and α configurations of the galactose units were deduced from the coupling constant values of the anomeric protons at δ_H 4.69 (*d*, *J* = 6.8 Hz, H-1′) and δ_H 5.53 (*d*, *J* = 3.1 Hz, H-1″). The HMBC correlations of H-1′ with C-3 and that of H-1″ with C-6′ confirmed a β-galactose linked to glycerol and α-galactose to C-6′. Alkaline hydrolysis with NaOMe–MeOH of **1** yielded methyl linolenate (methyl 9*z*,12*z*,15*z*-octadecatrienoate) and methyl palmitate (methyl hexadecanoate), which showed same retention times (t_R for methyl linolenate 9.79 min and t_R for methyl palmitate 8.85 min) on GCMS analysis as those of the standards (Sigma–Aldrich). As two peaks were observed in the GCMS analysis, it was concluded that two of the three fatty chains are identical. According to the molecular mass observed in the HRESIMS and the higher intensity of the peak related to methyl linolenate in the GCMS confirmed two linolenoyl and one palmitoyl units in compound **1**. The *Z*-geometry of the double bonds in the fatty acid units was supported by (a) absorption band observed at 721 cm^{-1} in the IR spectrum (about 967 cm^{-1} in case of trans double bond) (b) chemical shifts of the methylene carbons adjacent to the double bonds appeared at δ_C 26–28 in the ^{13}C NMR spectrum of **1**, while those for the *E*-geometry appear at δ_C 32–33 (Jung et al., 1996). Regio-selective enzymatic hydrolysis of **1**, using Lipase type XIII from *Pseudomonoas* sp. (liberating mostly the acyl moiety at C-1 of glycerol) afforded mostly palmitic acid (hexadecanoic acid), which was identified after methanolysis by GCMS analysis (t_R for methyl palmitate 8.85 min). Thus the palmitoyl at C-1 and the two linolenoyl units at C-2 and C-3″ were assigned. The configuration at C-2 was determined to be *S* by comparing the specific rotation sign $[\alpha]_D^{27}$ + 40.0 (*c*, 1.0, MeOH) with that of the identical glyceroglactolipids containing β-D-galactose at C-3 and α-D-galactose at C-6″ (Murakami et al., 1991; Reshef et al., 1997). Consequently, the structure of **1** was determined as (2*S*)-1-*O*-palmitoyl-2-*O*-linolenoyl-3-*O*-[α-D-galactopyranosyl-(1″ → 6′)-(3″-*O*-linolenoyl)-β-D-galactopyranosyl]glycerol.

Compound **2**, a colorless gum, displayed a quasimolecular ion in the negative HRESIMS at *m*/*z* 1211.7951 $[M + Cl]^-$, which corresponded to the molecular formula of $C_{67}H_{116}O_{16}$. Two extra hydrogen atoms in the molecular formula of **2**, as that of **1**, suggested the hydrogenation of one of the double bonds in **2**. The ^1H and ^{13}C NMR spectra of **2** were found to be identical to those of **1** except for resonances of one double bond begin absent in **2**. Similar to **1**, the sugars were identified as β-D-galactopyranose at C-1 and α-D-galactopyranose at C-6″. Alkaline hydrolysis with NaOMe-MeOH of **2** yielded methyl linolenate, methyl linoleate (methyl 9*z*,12*z*-octadecadienoate) and methyl palmitate, which were identified by GCMS analysis on having same retention times (t_R for methyl linolenate: 9.79 min, t_R for methyl linoleate: 9.75 min and t_R for methyl palmitate: 8.85 min) as those of the standards (Sigma–Aldrich). Regio-selective enzymatic hydrolysis of **2** afforded mostly palmitic acid, which was identified after methanolysis by GCMS analysis as a similar manner to that of **1** and helped in assigning the palmitoyl residue at C-1. The fragment ions at *m*/*z* 594 in the negative ESIMS spectrum supported the attachment of linolenoyl at C-2 of the glycerol and linoleoyl at C-3" of β-D-galactose (see Scheme 1).



Scheme 1. Fragments observed in ESIMS in (a) negative and (b) positive modes.

The quasimolecular ion observed in the negative HRESIMS of **3** at *m*/*z* 1187.7988 $[M + Cl]^-$ corresponded to the molecular formula of $C_{65}H_{116}O_{16}$. The ^1H and ^{13}C NMR spectra of **3** were identical to those of **1** and **2** except for the olefinic resonances counting for three double bonds in **3**. Alkaline hydrolysis of **3** with NaOMe–MeOH yielded methyl linolenate and methyl palmitate, which were identified by GCMS analysis as for **1**. One linolenoyl and two palmitoyl units were suggested in **3** according to the molecular mass observed in the HRESIMS. The palmitoyl residue at C-1 was supported by the regio-selective enzymatic hydrolysis of **3**, which liberate mostly palmitic acid, identified after methanolysis by GCMS analysis in a similar manner to that of **1**. Thus, it was concluded that one palmitoyl residue was attached to C-1. On the basis of the fragment ions at *m*/*z* 401, 573 and 591 observed in the positive ESIMS (see Scheme 1), linolenoyl at C-2 and palmitoyl at C-3″ moieties were established.